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Plasma Membrane Vesicles from Source and Sink Leaves[1]

Changes in Solute Transport and Polypeptide Composition

Rémi Lemoine, Olivier Gallet, Cécile Gaillard, Wolf Frommer, and Serge Delrot*

Laboratoire de Physiologie et Biochimie Végétales, Unité Associée Centre National de la Recherche Scientifique 574, Université de Poitiers, 25 rue du Faubourg Saint-Cyprien, 86000 Poitiers, France (R.L., O.G., C.G., S.D.); and Institut für Genbiologische Forschung, Ihnestrasse 63, D-1000 Berlin 33, Federal Republic of Germany (W.F.)

ABSTRACT

Plasma membrane vesicles (PMVs) were prepared by phase partitioning from microsomal fractions of either sink or source leaves of sugar beet (*Beta vulgaris* L.). The purity, the internal volume, the sidedness, and the sealingness of PMVs prepared from sink leaves did not differ from those measured with PMVs from source leaves. Yet, in response to an imposed proton motive force, PMVs from source leaves accumulated about 4-fold more sucrose than PMVs from sink leaves. The developmental stage did not affect the uptake of glucose and valine in PMVs prepared from leaf tissues. It was concluded that the sink/source transition is accompanied either by the incorporation into the plasma membrane of leaf cells of proteins mediating proton-sucrose cotransport, or by their activation. *N*-ethylmaleimide and a polyclonal ascitic fluid directed against the 42-kD region of the plasma membrane containing a putative sucrose carrier inhibited the uptake of sucrose in PMVs from source leaves, but not in PMVs from sink leaves. Sodium dodecyl sulfate gel electrophoresis and western blot suggested that the 42 polypeptide was more abundant in the PMVs from source leaves than in the PMVs from sink leaves.

The development of the leaves in plants is characterized by a transition from a heterotrophic stage in young leaves to an autotrophic stage in mature leaves. The different aspects of this topic have been recently reviewed (27). Young leaves import the sugars that they need for growth, whereas mature leaves will export the excess of sugar they produce to other part of the plants. Apart from its central role in leaf development, this transition provides a straightforward system to compare sink and source tissue without dealing with the complications introduced by the study of different organs (27). The sink/source transition of the leaf is a highly integrated process beginning when the leaf has reached about 35% final lamina length, and proceeds from the tip to the base (7). The transition is made possible by a series of changes affecting both metabolism and transport.

From the metabolic point of view, the cessation of import does not require achievement of positive carbon balance and is not the direct result of export initiation (26). In contrast, the beginning of export requires the development of metabolic processes able to provide sucrose for export. In sugar beet, accompanying the import-export transition is an increase in photosynthesis, an increase in the relative proportion of $^{14}CO_2$-derived soluble compounds, and a change in sugar partitioning favoring sucrose synthesis. This change is paralleled by the development of sucrose phosphate synthase activity rather than by a decrease of the sucrose-hydrolyzing enzymes (12). In the same material, phloem loading begins 35 to 45 h prior to onset of export, and it has been suggested that this triggers the initiation of export by increasing sufficiently the solute concentration within the conducting cells (7).

In sugar beet, sucrose is the major form of carbon transport. Whereas sucrose is loaded in the phloem from the apoplast in source leaves (11), it is unloaded symplastically from the phloem in sink leaves (23, 24). In connection with this change in the pathway of sucrose transport, studies of plasmodesmatal frequencies in broad bean (another species where loading is clearly apoplastic) have shown that the maturation of the leaf is characterized by a marked symplastic isolation of the phloem and, within the phloem itself, by the isolation of the conducting complex (3). Apoplastic loading requires at least two carrier-mediated steps for sucrose transport, i.e. exit from the mesophyll cell and uptake into the conducting complex. Recent progress in the preparation of PMV[2] and in the energization of these vesicles by artificial gradients (4–6, 13, 14) now makes it possible to study sucrose transport directly at the membrane level. In the present study, we have used these techniques, as well as one-dimensional gel electrophoresis, to compare the ability of PMVs prepared from importing or exporting leaves to take up sucrose actively and to study associated changes in the protein pattern.

MATERIALS AND METHODS

Preparation of PMV

Growth of plants, preparation of PMVs by phase partitioning, and assays for marker enzymes were conducted as de-

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[2] Abbreviations: PMV, plasma membrane vesicles; NEM, *N*-ethylmaleimide; pmf, proton motive force; ΔpH, transmembrane pH gradient; $\Delta\psi$, transmembrane electrical gradient; TPP, tetraphenylphosphonium bromide; DMO, dimethyloxazolidine dione.

scribed in Gallet et al. (10). Source leaf vesicles were prepared from fully expanded leaves (about 25 cm long), whereas sink leaf vesicles were prepared from growing leaves that were less than 4 cm long. Two millimolar DTT and 5 mM EDTA were included in the grinding buffer (13), and the vesicles were equilibrated and stored as a concentrated protein suspension (10–15 mg protein·mL^{-1}) in buffer K (280 mM sorbitol, 0.5 mM $CaCl_2$, and 0.25 mM $MgCl_2$ buffered at pH 7.5 with 50 mM K-phosphate) in the presence of 1 mM EDTA and 0.5 mM DTT.

Solute Uptake Experiments

The procedures used for the energization of the PMVs and for their filtration have been detailed previously (13). The uptake solutions contained 1 mM sucrose plus 41 kBq·mL^{-1} [6,6′(*n*)-^{3}H]sucrose and 1 mM glucose plus 11 kBq·mL^{-1} D-[U-^{14}C]glucose. Parallel experiments were run on the uptake of 1 mM [^{3}H]valine (41 kBq·mL^{-1}). For uptake in nonenergized conditions (no gradient), the incubation medium was buffer K, in which vesicles had been equilibrated before storage. For uptake in energized conditions, the concentrated vesicles solution was first preincubated for 30 min with 5 μM valinomycin. The incubation medium was the same as for experiments in nonenergized conditions, except that it was buffered at pH 5.5 with 50 mM Na-phosphate buffer containing 5 μM valinomycin. The combination of a 50 mM K-phosphate buffer (pH 7.5) inside the vesicles with a 50 mM Na-phosphate buffer (pH 5.5) in the incubation medium generates a ΔpH (outside acidic) and a K^+ diffusion potential, resulting in a $\Delta\psi$ (inside negative). The results are expressed as the difference between the uptake in energized conditions ($\Delta pH + \Delta\psi$) and the uptake in nonenergized conditions (no gradient).

The sensitivity of uptake to 1 mM NEM was tested in the presence of 0.5 mM DTT, as described previously (14), after a 30-min preincubation of the vesicles with NEM. The effect of a polyclonal anti-42 kD ascitic fluid on sucrose uptake was tested by preincubating the vesicles (1 mg protein·mL^{-1}) for 45 min with the ascitic fluid (0.1 mg immunoglobulin·mL^{-1}) in a total volume of 200 μL of buffer K. Control vesicles were preincubated with 0.1 mg·mL^{-1} control immunoglobulins already tested for their lack of effect on sucrose uptake by PMV. Both sets of vesicles were then diluted to 2 mL with buffer K, equilibrated for an additional 30 min, and centrifuged for 40 min at 150,000*g*. The pellets were resuspended in 20 μL of buffer K and used for uptake experiments.

Determination of the Internal Volume of the Vesicles

The internal volume of the vesicles was determined by the equilibration method described by Rottenberg (22) using ^3H_2O and [^{14}C]dextran as respective markers for the water space and extravesicular space. The procedure is as detailed in Lemoine et al. (13), except that the vesicles (1 mg protein·mL^{-1}) were incubated in K-buffer, pH 7.5, containing 69 kBq·mL^{-1} ^3H_2O and 42 kBq·mL^{-1} [^{14}C-carboxyl]dextran (mol wt 50,000–70,000) instead of [^{14}C]sorbitol. The osmotic space was determined from the variations of the water and extravesicular spaces at different osmolalities of the medium adjusted with sorbitol in 50 mM K-phosphate buffer.

Determination of ΔpH and $\Delta\psi$

The two components of the pmf were determined simultaneously by incubating the vesicles as described above for sugar uptake experiments, except that sugar in the incubation medium was replaced with 4 μM [^{3}H]TPP (4.8 TBq·mL^{-1}) to evaluate $\Delta\psi$ and 7 μM [^{14}C]DMO (2.0 TBq·mL^{-1}) to evaluate ΔpH. For experiments on [^{3}H]TPP uptake, cellulose acetate filters (Sartorius) were used instead of the HAWP filters (Millipore) normally used for sugar uptake, because TPP binds unspecifically to the HAWP filters (13). Procedures for uptake and conversion of the uptake values of the probes to the corresponding gradients were as described in Lemoine et al. (13).

SDS-PAGE and Immunoblotting

The membrane proteins were solubilized and separated by SDS-PAGE according to Gallet et al. (10) with a stacking gel of 4.75% acrylamide and a separating gel containing a linear gradient of 10 to 22% acrylamide. Solubilization of the membrane proteins was conducted at 37°C for 30 min in the presence of 1 mM PMSF and 1 mM EDTA. Immunoblotting was conducted as described in Lemoine et al. (15), except that the 16-h incubation of the nitrocellulose paper in PBS was omitted and the blocking solution was composed of 5% defatted milk and 0.2% Tween 20 in PBS. Polypeptides were tested for their reactivity with a polyclonal ascitic fluid raised in mouse according to the immunization schedule described by Turano et al. (25). The ascitic fluid was raised against the 42-kD polypeptide of PMVs from source leaves previously shown to be involved in the uptake of sucrose (9, 15, 17). This polypeptide was electroeluted from SDS-PAGE gels and used to immunize mice.

Other Methods

The ATPase activity was measured as described in Bennett et al. (2), with an optimal Triton X-100 concentration of 0.02% (w/v). When inhibitors of the ATPase were tested, vesicles were preincubated for 15 min in their presence. The H^+-pumping ATPase activity was measured with acridine orange according to Palmgren and Sommarin (19). In both experiments, ATP and $MgCl_2$ were used at 3 mM. Protein was determined according to Bearden (1) with BSA as a standard.

RESULTS

PMVs from sink and source leaves were characterized for their purity, their integrity, for various parameters involved in solute uptake, for their ability to take up actively sugars and amino acids, and for their polypeptide composition.

Isolation of PMVs from Sink and Source Leaves

PMVs from sink and source leaves were purified from the corresponding microsomal fractions by phase partitioning in a mixture of dextran T 500 and PEG 3350. The same polymer concentration (6.5%) was used for both kinds of leaves. Starting from 50 g fresh weight of leaf tissue, the amounts

Table I. *Comparison of H^+-Pumping and ATPase Activities in PMV from Sink and Source Leaves*

The results are means of three determinations made on independent preparations. ATPase assays were run in the presence of 0.02% (w/v) Triton X-100 for the inhibitors listed. The results in the parentheses give the percentage of inhibition compared to the control. H^+ pumping was studied after four freeze-thaw cycles.

	PMV Origin			
	Sink leaves		Source leaves	
ATPase activity (nmol Pi·min^{-1}·mg^{-1} protein)	252		228	
+250 μM sodium vanadate	14	(95)	22	(90)
+100 μM sodium molybdate	272	(−8)	229	(0)
+50 mM sodium nitrate	232	(8)	205	(10)
+100 μM sodium azide	282	(−12)	269	(−15)
H^+ pumping (−Δ optical density·min^{-1}·mg^{-1} protein)	0.22		0.26	

of plasma membrane proteins recovered were 3.0 and 2.6 mg for sink and source leaves, respectively. Both types of preparations were similar in purity, as shown by the study of ATPase activity in the presence of specific inhibitors (Table I). Vanadate, a powerful inhibitor of the plasma membrane ATPase, drastically reduced the ATPase activity in both type of vesicles. Only nitrate also inhibited the ATPase activity, suggesting a slight contamination of the preparations with tonoplast vesicles, never exceeding 10%. Measurements of the ATPase latency in the presence of 0.02% Triton X-100 showed that vesicles from both origins were also similar in orientation (73% of right-side out vesicles in sink leaf vesicles and 76% in source leaf vesicles). The H^+-pumping activity could be monitored in vesicles from both origins, confirming the tightness of the vesicles (Table I). Considering either the pumping of protons or the breakdown of ATP (Table I), the specific activities of the enzyme were rather close in PMVs from sink leaves and from source leaves.

Comparison of Some Parameters Relative to Sucrose Uptake in PMVs from Source and Sink Leaves

The internal volume of the vesicles was measured with ^3H_2O and [^{14}C]dextran at different osmolalities. This technique allows the precise determination of the osmotic space of the vesicles by preventing artefacts in the measurements (adsorption, nonspecific binding, unstirred layers) (13, 21). These artifacts are apparent when the volume corresponding to tritiated water and the volume corresponding to dextran are studied as a function of the inverse of the medium osmolality. This allows the extrapolation of both volumes to infinite osmolality where the two volumes should be equal to zero. If both volumes are not equal to zero at infinite osmolality for any of the reasons already mentioned, the actual osmotic volume can be calculated by correcting the values of the dextran space with the difference between the dextran space and the water space at infinite osmolality (21).

The two curves in Figure 1 have been corrected for this difference at infinite osmolality, as detailed in Lemoine et al. (13). [^{14}C]Dextran was preferred to [^{14}C]sorbitol used in our previous experiments with PMVs from source leaves. Indeed, it was not possible to measure the osmotic volume in vesicles from sink leaves with the latter compound, probably because the plasma membrane from sink leaves was permeable to sorbitol. Similar conclusions were drawn from data obtained with PMVs isolated from broad bean leaves (18). Figure 1 shows that the osmotic volume of vesicles from sink leaves was more sensitive to the changes of osmolality than that of source leaves. At the osmolality used in the experiments on sugar uptake (410 mOsm, arrow in Fig. 1), the internal volume was 2.8 μL·mg^{-1} protein for source leaf vesicles and 3.5 μL·mg^{-1} protein for sink leaf vesicles. The value obtained here with [^{14}C]dextran (2.8 μL·mg^{-1} protein) in PMVs from source leaves is in good agreement with the value obtained on the same material with [^{14}C]sorbitol (2.2 μL·mg^{-1} protein [16]).

These values of internal volume were used to calculate ΔpH and Δψ from the uptake of the probes DMO and TPP, respectively. Table II shows that ΔpH was higher in PMVs from sink leaves, whereas Δψ was slightly higher in PMVs from source leaves. Consequently, the pmf calculated from these values was slightly higher in PMVs from sink leaves than in PMVs from source leaves (−211 and −189 mV, respectively).

Active Uptake of Sucrose, Glucose, and Valine

Active uptake of sucrose was higher in PMVs isolated from source leaves than in vesicles from sink leaves (Fig. 2, top). After 1 min of incubation, the amount of sucrose accumulated

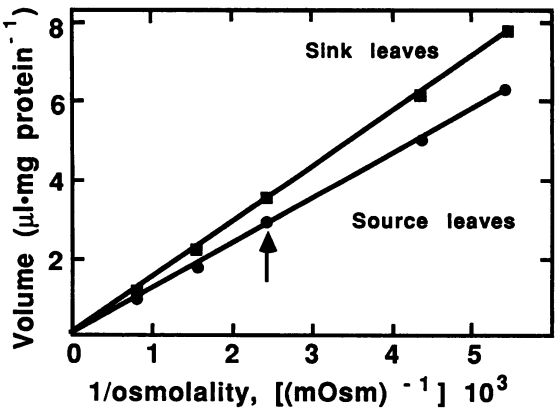

Figure 1. Osmotic volume of PMVs from source (●) and sink (■) leaves as a function of the inverse of the osmolality of the medium. The osmotic volume was calculated as the difference between the water space measured with ^3H_2O and the extravesicular space measured with [^{14}C]dextran. Results are the mean of four replicates made on two different PMV preparations.

Table II. *Measurements of the Components of the pmf (ΔpH + Δψ) in PMV's Isolated from Source or Sink Leaves*

ΔpH and Δψ have been calculated from the uptake values after a 2-min incubation of the probes DMO and TPP, respectively, and the pmf computed from the corresponding values. The results are means of eight independent measurements made on two different preparations for each type of vesicle and have been corrected for the passive uptake of the probes measured in the absence of gradients.

	Origin of the PMVs	
	Sink leaves	Source leaves
ΔpH (unit)	1.4	0.9
Δψ (mV)	−127	−141
pmf (mV)	−211	−189

in vesicles from source leaves was about 4-fold higher than the corresponding value in vesicles from sink leaves. The shapes of the two curves were quite different. In vesicles from source leaves, the accumulation of sucrose was only transient, whereas the shape of the uptake curve for sink leaves vesicles was smooth, with no significant difference between 2 and 10 min.

N-ethylmaleimide and a polyclonal ascitic fluid directed against the 42-kD region of the plasma membrane containing

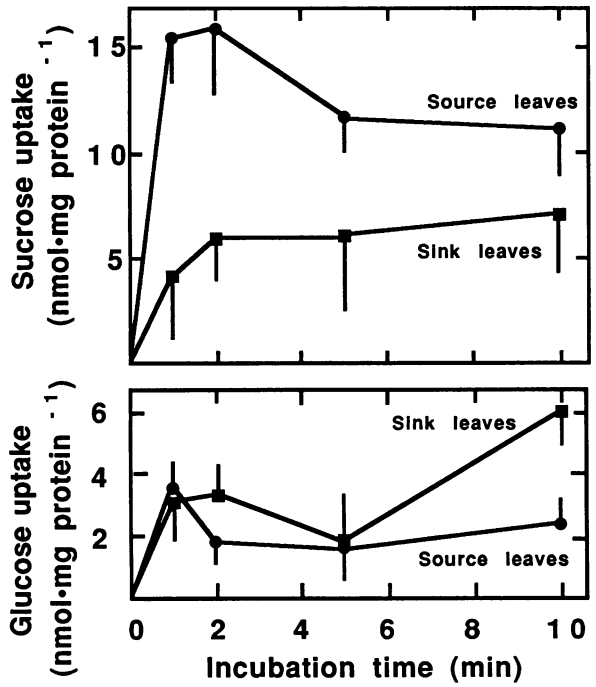

Figure 2. Active uptake of 1 mM [^{3}H]sucrose (top) and 1 mM [^{14}C]-glucose (bottom) as a function of time in PMVs from source (●) and sink (■) leaves. The uptake of sucrose and glucose were studied on the same samples. Results are expressed as the uptake in the presence of ΔpH + Δψ minus the uptake in the absence of gradients. The results are the mean ± SD of eight replicates made on two different PMV preparations.

a putative sucrose carrier inhibited the uptake of sucrose in PMVs from source leaves, but not in PMVs from sink leaves (Table III).

To investigate whether the sink to source transition led to a difference in the type of sugars taken up by the cells, the uptake of sucrose in vesicles from sink or source leaves was also compared with the uptake of glucose (Fig. 2, bottom). In PMVs from source leaves, the uptake of glucose was much lower than the uptake of sucrose, whereas in PMVs from sink leaves, glucose uptake was similar to sucrose uptake. There was no significant difference between the active uptake of glucose in vesicles from sink leaves and from source leaves. Given the internal volumes of the vesicles (2.8 $\mu L \cdot mg^{-1}$ protein for source leaf vesicles and 3.5 $\mu L \cdot mg^{-1}$ protein for sink leaf vesicles, see above), it may be calculated that glucose uptake did not occur against a concentration gradient in PMVs from sink leaves or from source leaves, at least for short incubation times. The only value that was significantly above diffusion equilibrium was that measured in PMVs from sink leaves incubated for 10 min.

Therefore, it was interesting to investigate the uptake of valine, which is actively taken up by PMVs in response to an imposed pmf (8, 16). The initial rate of valine uptake was the same in PMVs from sink and source leaves, but PMVs from source leaves seemed to be able to sustain the valine gradient for a longer time than the PMVs from sink leaves (Fig. 3).

Overall, the uptake data show that the initial rate of sucrose uptake is higher in PMVs from source leaves than in PMVs from sink leaves, whereas the uptake of glucose and of valine was not affected by the developmental stage of the leaves. The component of sucrose uptake that appears in mature leaves is sensitive to NEM and to a polyclonal anti-42 kD ascitic fluid. This led us to compare the polypeptide pattern of sink and source PMVs.

SDS-PAGE and Immunoblotting

A comparison between vesicles from source and sink leaves was made on the polypeptide patterns observed after SDS-

Table III. *Effect of NEM and anti-42 kD Ascitic Fluid on the Active Uptake of Sucrose by PMVs from Sink and Source Leaves*

The results are expressed as percentage of the control value. The actual uptake values are given inside the parentheses as nmol sucrose (mg protein)$^{-1}$. Duration of uptake was 2 min. The results are the difference ± SE between the uptake in energized conditions and the uptake in nonenergized conditions and are from one[a] or two[b] experiments (four replicates per experiment). Values differing significantly ($P < 0.05$) from the control are indicated with an asterisk.

	Control	+ NEM (1 mM)	+anti 42-kD Ascitic Fluid (0.1 mg immunoglobulin mg^{-1} protein)
Source leaves	100%	37%	42%
	(13.5 ± 0.5)[b]	(4.9 ± 0.9)[b]	
	(24.5 ± 3.2)[a]		(10.3 ± 2.1)[a]*
Sink leaves	100%	87%	87%
	(6.2 ± 0.8)[b]	(5.4 ± 1.4)[b]	
	(6.2 ± 1.8)[a]		(5.5 ± 1.4)[a]

* $P < 0.05$, significantly different from control.

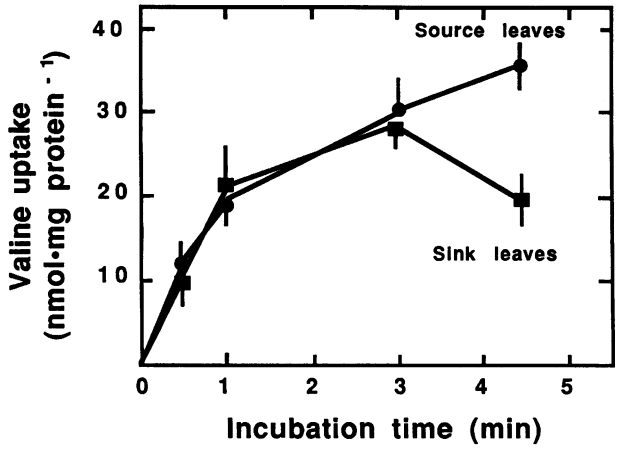

Figure 3. Active uptake of 1 mM [^{3}H]valine as a function of time in PMVs from source (●) and sink (■) leaves. The results are the mean ± SD of four replicates from one typical experiment.

PAGE. The reactivity of the membrane polypeptides with antibodies directed against a 42-kD NEM-binding polypeptide previously reported to be involved in the uptake of sucrose (9, 10, 15, 17) was also tested.

The polypeptide profiles of plasma membrane proteins isolated from source and sink leaves are presented in Figure 4, lanes A and B, respectively. The two profiles look rather similar, but a careful examination shows that the following bands are only present in source leaves: one at 55 kD, a complex of two to three bands in the 42-kD region (see

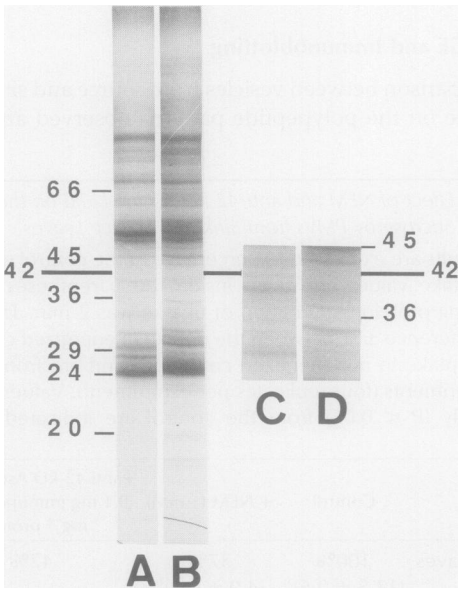

Figure 4. SDS-PAGE of PMVs from source (A, C) and sink (B, D) leaves. One hundred-microgram proteins were deposited in each lane and stained with Coomassie blue. The position of the molecular mass standards is shown on both sides. Lanes C and D show higher magnifications of the 30- to 45-kD regions of lanes A and B, respectively.

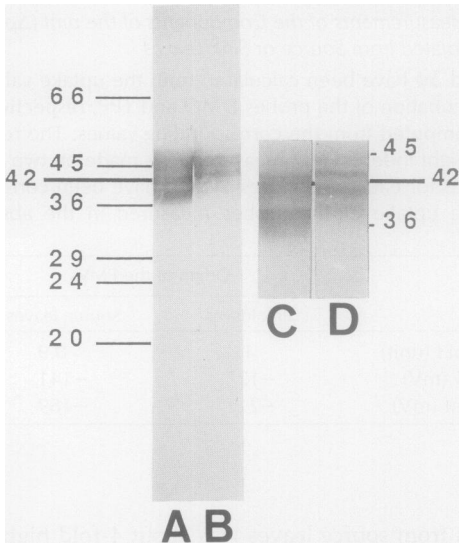

Figure 5. Immunoblots of PMVs from source (A, C) and sink (B, D) leaves. One hundred micrograms of PMV protein were separated by SDS-PAGE, transferred to nitrocellulose, and incubated with anti-42 kD ascitic fluid (1/500 dilution). A and B are blots from one experiment, and C and D are blots from another experiment run with different batches of plasma membrane.

below), one band at 36 kD, and one around 29 kD. On the contrary, the following bands are only present in sink leaf vesicles: 107 kD, diffuse bands around 62, 35, 33, and 31 kD. Some other bands differ only in intensity between the two lanes. A higher magnification of the 42-kD region (Fig. 4, lanes C and D) showed that two bands (about 43.5 and 42 kD) are clearly absent in sink leaves. A third one at 41 kD is fainter.

Figure 5 presents immunoblots of plasma membrane from source and sink leaves after incubation with the anti-42 kD mouse polyclonal ascitic fluid inhibiting sucrose uptake in source leaves (see Table III). The breadth of the band reacting in lane A (source leaves) from 37 to 50 kD is certainly due to the procedure used for the preparation of the antigen used for the immunization (see "Discussion"). However, there was no cross-reaction with other polypeptides. The 42-kD region was clearly stained in lane A, whereas in lane B, no strong reaction can be seen with polypeptides of molecular mass lower than 43 kD. Using the same ascitic fluid, the pattern observed varied slightly depending on the membrane preparation, and another example is shown in Figure 5, lanes C and D. Discrete bands can be seen in this blot, and there is clearly one band reacting at 42 kD in source leaves (Fig. 5, lane C) that is completely absent in sink leaves (Fig. 5, lane D). Therefore, despite the variations in the patterns observed, the 42-kD region was always much less reactive in PMVs from sink leaves than in PMVs from source leaves.

DISCUSSION

Young immature leaves are net importers of assimilates and begin to export only after maturation. The major components exported from source leaves are carbohydrates, in

the form of sucrose, and amino acids (20). During their development, leaves thus undergo an inversion with respect to the direction of transport. In mature spinach leaves, the concentration of the amino acids in the sieve sap is in the same range as the concentration of the amino acids in the cytosol of the mesophyll cells. In contrast, the concentration of sucrose has been estimated to be 10-fold higher in the sieve sap than in the cytosol of the mesophyll cells (20). Therefore, it may be assumed that the sink/source transition is accompanied by the synthesis or the activation of protein machinery able to concentrate sucrose in the sieve tubes. These changes are expected to become visible at the plasma membrane. To analyze these changes, we have isolated PMVs from sink and source leaves and studied their transport characteristics and their protein pattern.

PMVs from sink and source leaves were comparable in purity (Table I) and size (Fig. 1). The activity of the plasma membrane proton-pumping ATPase was also studied as a marker of the functional integrity of the membrane. There was no difference in the activity of this enzyme in PMVs from sink and source leaves based on the measurement of ATP hydrolysis or H^+-pumping (Table I). Assuming that the initial specific activities were similar in the corresponding microsomal fractions from young and exporting leaves, these data suggest that the PMVs from sink leaves were not preferentially degraded during their preparation compared to the PMVs from source leaves. Furthermore, the pmf measured upon imposition of an artificial electrochemical gradient was also similar for both kinds of PMVs (Table II). Therefore, the PMVs from sink and source leaves did not differ in their sealingness.

Yet, proton-driven uptake of sucrose was lower in PMVs from sink leaves than in PMVs from source leaves (Fig. 2). Given the internal volume of the vesicles, PMVs from source leaves were able to accumulate sucrose to a ratio of 6 after 1 min of incubation (Fig. 2, top), whereas this ratio was only 1.3 in PMVs from sink leaves. Importantly, the uptake component that develops during the sink/source transition is sensitive to NEM and to the anti-42 kD ascitic fluid (Table III). These data further confirm the NEM sensitivity of sucrose uptake in PMVs from mature sugar beet leaves (14). Although this sensitivity has been questioned (5), detailed experiments conducted in our laboratory show that the observed discrepancy may be simply explained by different experimental procedures (S. Sakr and S. Delrot, in preparation). The sensitivity of sucrose uptake to the anti-42 kD polyclonal serum also confirms data obtained with other anti-42 kD sera on sucrose uptake by protoplasts (15) and by PMVs (9). Furthermore, the present data show that the component of uptake that is sensitive to NEM and to the anti-42 kD polyclonal ascitic fluid is incorporated (or activated) during the sink/source transition.

The strong sucrose uptake observed in PMVs is in marked contrast with the values found for glucose uptake, which are similar in PMVs from source and sink leaves. Low uptake of glucose in vesicles from mature leaves had already been mentioned (4, 14). Low levels of glucose uptake in PMVs from sink leaves suggest that glucose is not a form of uptake across the plasma membrane for imported sugar. This conclusion also fits well with the results of Giaquinta (11), who showed that sucrose was not cleaved prior to its uptake in cells from sink leaves, despite the presence of an acid invertase.

The conclusion that increased sucrose uptake in PMVs from source leaves is not due to a higher pmf is confirmed both by measurements of the pmf (Table II) and by the fact that the initial rates of valine uptake (also energized by the pmf; 8, 16) were the same in sink leaf PMVs and in source leaf PMVs (Fig. 3).

These results and the fact that sucrose uptake is carrier mediated in PMVs from source leaves may be interpreted either as an incorporation of sucrose carriers in the plasma membrane of leaf cells during the sink/source transition or as an activation (or derepression) of carriers preexisting in the membrane of sink cells. A third possibility to explain a specific increase in sucrose uptake during the sink/source transition could be a differential recovery of transport-competent vesicles. Indeed, since all the parameters measured (uptake, sealingness, sidedness) concern a heterogeneous population of vesicles derived from mesophyll, phloem, and xylem cells (13), it cannot be excluded that increased sucrose uptake may be due to a differential recovery of a population of transport-competent vesicles. However, the latter possibility cannot be tested at the present time.

Although it is difficult to discard the ideas of carrier activation or of differential recovery of vesicles, our results rather support a mechanism leading to synthesis and incorporation of carriers during the sink/source transition. Former results from this laboratory have led to the identification of a 42-kD polypeptide as a putative sucrose carrier (9, 10, 15). In the present work, a polyclonal ascitic fluid raised against the 42-kD region of the plasma membrane of source leaves from sugar beet was used to probe PMVs from sink and source leaves by immunoblot. This ascitic fluid, which inhibited the uptake of sucrose in PMVs from source leaves (Table III), was used successfully to identify plasma membrane fractions that exhibited sucrose transport activity when reconstituted into proteoliposomes (17). The appearance of a 42-kD band in PMVs from source leaves (Figs. 4 and 5), together with a specific increase of sucrose transport (Figs. 2 and 3), and the sensitivity of this transport component to a polyclonal anti-42 kD ascitic fluid (Table III) may lend indirect, but additional support to the idea that a 42-kD polypeptide is involved in sucrose transport, and would suggest that this polypeptide is incorporated in the membranes during leaf development. Yet, the relatively poor specificity of the anti-42 kD serum used (due to the procedure used for antigen preparation, see discussion in refs. 9 and 15) precludes a precise identification of the polypeptidic band concerned.

The biochemical approach presented here can also be related to the present physiological background concerning the changes in sucrose transport during the sink/source transition in leaf. During the maturation of the leaf, the conducting complex (sieve tube/companion cell) becomes isolated from the surrounding cells because of the progressive closure of plasmodesmata (3, 26) preventing the symplastic unloading of sucrose. The weak activity of sucrose transport in PMVs from sink leaves is in agreement with a symplastic pathway for sugar import in young leaves. According to Fellows and Geiger (7), the initiation of phloem loading and

the build-up of the solute concentration in the conducting complex are critical events in the onset of export. The appearance of sucrose transport activity during the sink to source transition is therefore necessary for the loading of this sugar into the phloem and for the beginning of export. Likewise, after closing of the plasmodesmata, it is likely that carrier proteins are necessary to mediate export of sucrose from the mesophyll cells to the apoplast, where it will be loaded into the phloem. The data presented here clearly show that the proton-driven accumulation of sucrose is higher in PMVs from source leaves than in PMVs from sink leaves and suggest that this may be related to a higher number of sucrose carriers. However, because the vesicles used in the present experiments, either from source or sink leaves, were isolated from whole leaves, it is not possible to consider the uptake of sucrose recorded in PMVs as only representative of the loading of the phloem cells (4, 13). The development of sucrose uptake in PMVs during the sink/source transition, therefore, may be due to a system allowing either a better retrieval of the assimilates in the mesophyll cells, or to a system mediating phloem loading of sucrose into the conducting complex.

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